Engineering an HIV-1 Vector to Express an Epitope-tagged Vif Protein

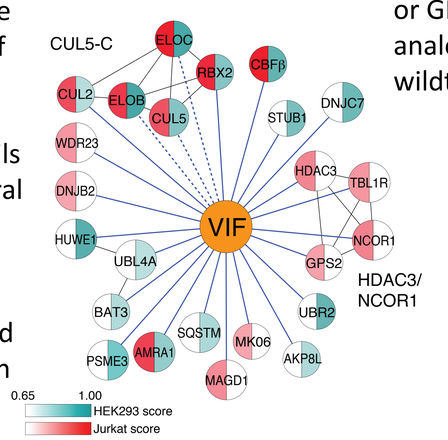
Andrew Clingerman
University of Minnesota
Undergraduate Research Opportunities program

Does HIV-1 Vif have any unique protein-protein interactions in a replicating HIV infection?

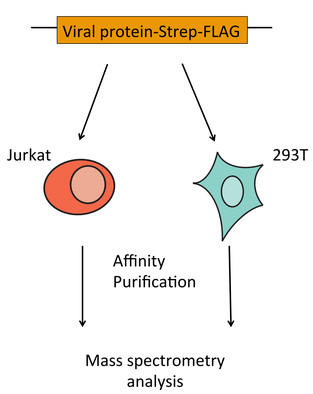
HIV-1 proteins form numerous complex interactions with human proteins over the course of an HIV-1 infection[1].
The purpose of this study has been to develop a tool to aid in elaborating on the group of the protein interactions surrounding the HIV protein Vif that have been described previously.

Background and Previous work

Vif is an HIV protein that functions to disarm the innate immune response against HIV by restricting a group of proteins known as the APOBEC3 enzymes[2]. These enzymes catalyze the conversion of cytosines to uracils in cDNA synthesized during retroviral replication, thus lowering the infectivity of the retroviruses[3].

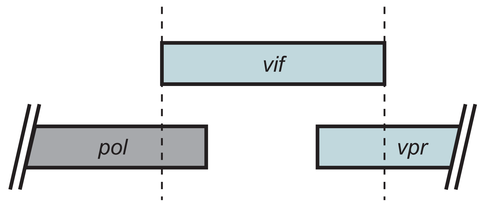


Previous studies that have identified the human proteins interacting with Vif have used mass-spectrometry analysis of immunoprecipitated Vif complexes after having overexpressed Vif in immortalized and transformed human cell lines, such as 293T and Jurkat[4,5].

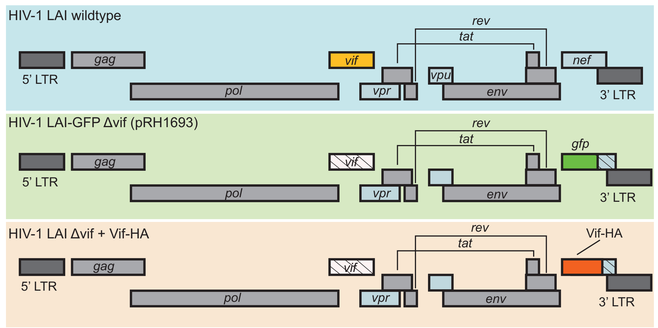


Although these studies have pointed out several crucial interactions, I hypothesize that there are novel protein interactions with Vif during a true HIV-1 infection in H9 or in Primary CD4+ T cells.

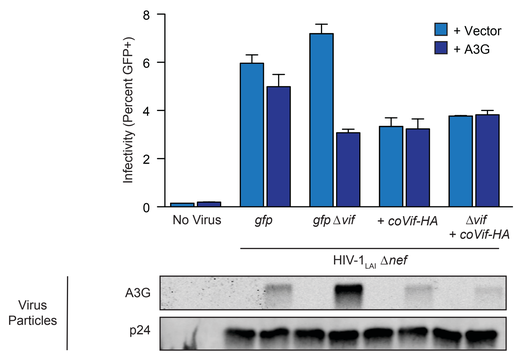
Research and Results



The *nef* reading frame of the virus was used to encode the epitope tagged Vif protein. The wildtype vif sequence overlaps with *pol* at the 5' end and *vpr* at the 3' end, so attaching an epitope tag to the end of the wildtype protein would have been challenging. Previous studies have also shown that the Nef protein is dispensible in most cell cultures. Two constructs encoding either an epitope tagged vif or GFP in the *nef* reading frame were produced as well as two analogous constructs with two premature stop codons in the wildtype vif sequence. The epitope tag chosen was the HA tag.



The viral constructs above were transfected into 293T cells and their infectivity was measured through the CEM GFP reporter cell line. Protein levels of the cells were also measure via western blot.



Discussion

The infectivity assays of the viral constructs are promising because they show similar levels of infectivity for cells transfected with a plasmid encoding APOBEC3G and a vector control. This indicates that the epitope tagged Vif protein is still fully capable of degrading APOBEC3G.

The western blots of the viral particles show precisely what was expected in that there are low levels of APOBEC3G being packaged into both the viral constructs and the wildtype GFP virus. This is consistent with infectivity levels observed previously.

The tests performed on the viral constructs seem to indicate that they are functional viruses. These tools will be significant in developing a more representative model of protein interactions between HIV and host proteins.

Acknowledgements

Many thanks go to my Graduate Student mentor, Brett Anderson, and my Principal Investigator, Dr. Reuben Harris, for providing me with such a unique opportunity to become involved in research.

Sources

1. Jäger, S. et al. Global landscape of HIV-human protein complexes. *Nature* 481, 365-370 (2012).
2. Harris, R. S., Hultquist, J. F. & Evans, D. T. The restriction factors of human immunodeficiency virus. *Journal of Biological Chemistry* **287,** 40875–40883 (2012).
3. Refsland, E. W. & Harris, R. S. in *Current Topics in Microbiology and Immunology* **371,** 1–27 (Springer Berlin Heidelberg, 2013).
4. Yu, X. *et al.* Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science* **302,** 1056–1060 (2003).
5. Jäger, S. *et al.* Vif hijacks CBF-β to degrade APOBEC3G and promote HIV-1 infection. *Nature* (2011). doi:10.1038/nature10693
Vif interactome map taken from Jäger, S. *et al.* Vif hijacks CBF-β to degrade APOBEC3G and promote HIV-1 infection. *Nature* (2011).